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Piperazine as a Linker for Incorporating the Nitric Oxide-releasing Diazeniumdiolate Group into Other Biomedically Relevant Functional Molecules

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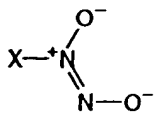
Synthetic procedures have been devised to exploit the bifunctional amine piperazine (pip) as a linker capable of attaching the nitric oxide (NO)-releasing diazeniumdiolate functional group $[N(O)NO]^-$ to a diverse selection of biomedically useful molecules. One of the amino groups bears the diazeniumdiolate, which may be substituted on oxygen as necessary to control its dissociation to NO, while the other is used to provide a site suitable for covalent bonding to the molecule requiring NO donor capability. *N,N'*-Disubstituted piperazines of the structure R-pip-N(O)=NOE were prepared either by using the nucleophilic character of the amino group or by converting it into an electrophilic moiety for reaction with nucleophilic centers in the molecules to be derivatized. Examples are reported in which E = CH_3 and R is bound to the *N'*-nitrogen: via amide linkages to the carboxyl groups of the drug ibuprofen and the amino acid derivative *N*-acetylmethionine; through a urea grouping to the ϵ-amino group of a protected lysine; via a carbamate linkage to poly(ethylene glycol); and by replacing the NH_2 nitrogens of nicotinamide and adenosine. Synthesis of analogues in which E = vinyl has been facilitated by introduction of $BrCH_2CH_2OSO_2Cl$ as a novel, efficient bromoethylating agent. Spontaneous NO releasers in the diazeniumdiolated piperazine series include both a fluorescent anion of half-life 5.5 min in which E = Na and R = dansyl and an "acetal" (E = CH_3OCH_2, R = H) whose half-life for NO release is approximately one month. The latter agent has made possible the conversion of poly(vinyl chloride) and phosphatidylethanolamine to NO-releasing derivatives. This chemistry should allow introduction of diazeniumdiolate groups into a wide variety of natural products, drugs, polymers, and other molecules whose activities could be beneficially combined with the ability to generate NO for biomedical applications.

Introduction

Nitric oxide (NO) plays a critical role in a variety of bioregulatory processes, including normal physiological control of blood pressure, neurotransmission and macrophage-induced cytostasis and cytotoxicity.[1] NO has limited solubility in water and it is unstable in the presence of various oxidants. This makes it difficult to introduce it as such into biological systems in a controlled or specific fashion. Consequently, the development of chemical agents that release nitric oxide (NO) is important if we are to target its bioeffector roles to specific cell types for pharmacological applications.[2-6]

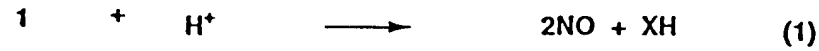
Drago[7] introduced complexes of structure **1** formed by the reaction of nucleophiles, including amines, with nitric oxide; Hrabie et al.[3] have extended the available library of amine/NO complexes to the polyamine series **2**. These diazeniumdiolate-containing compounds are advantageous agents for many studies requiring the controlled, gradual release of nitric oxide, since they are capable of regenerating nearly two equivalents of NO upon dissolution in neutral media.[8] The half lives, $t_{1/2}$, of these complexes range from 1.3 min to 1200 min at 37 °C in pH 7.4 phosphate buffer. The decomposition to NO is a spontaneous, first order reaction at constant pH (eq 1).[9]



1

$R^1N[N(O)NO]^-(CH_2)_XNH_2^+R^2$

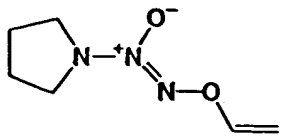
2



We have been interested in reacting amine/NO complex ions of structure **1** ($X = R_2N$) with alkylating agents (E^+) to provide stable, covalently bonded adducts, **3** (eq 2). Compounds of this type could serve as prodrugs that cannot release nitric oxide until they are metabolically reconverted to **1** by enzymes specific to the desired target cell type. Anions of structure **1** ($X = R_2N$) proved reactive toward a variety of electrophiles, such as alkyl halides, sulfate esters and epoxides, as in eq 2,[2] to give compounds of type **3**.

3

We recently reported the successful application of this strategy to the design of a liver-selective NO donor, **4**.[10] Liver enzymes metabolize this compound to give a diazeniumdiolate ion (**1**, X = pyrrolidin-1-yl) that spontaneously and rapidly ($t_{1/2}$ = 3 s) decomposes to NO at physiological pH. The administration of **4** to rats resulted in the blocking of tumor necrosis factor-α-induced apoptosis and hepatotoxicity, thus illustrating the potential utility of O^2-substituted diazeniumdiolates for targeting NO delivery in vivo.



4

Besides this prodrug approach, we have shown that polymers to which the $[N(O)NO]^-$ group is attached can serve as localized sources of nitric oxide.[11] For example, cross-linked poly(ethylenimine) exposed to NO inhibited the in vitro proliferation of rat aorta smooth muscle cells and showed potent antiplatelet activity in arteriovenous shunts placed in the baboon circulatory system.[11]

This paper introduces an alternate drug delivery strategy in which the diazeniumdiolate group can be bound via a linker moiety to a variety of substrates, thus furnishing a convenient means of adding the capacity for NO release to the substrate's other useful functional characteristics.

Piperazine as a linker in diazeniumdiolation reactions. Piperazine, a bifunctional base used medicinally for its anthelmintic properties, appeared to us to be the ideal type of compound for these studies. Early reports of the high pressure reaction of piperazine with NO show that various salts of diazeniumdiolated piperazines can be produced.[7,12] Hrabie et al.[13] took advantage of the solubility properties of monodiazeniumdiolated piperazine to isolate the anion as the sodium salt in its pure form. We aim, with the assistance of protection and deprotection techniques, to use one of piperazine's two nucleophilic sites for the reaction with nitric oxide while the second nitrogen remains available for linking the diazeniumdiolated piperazine to a desired substrate. The examples described below illustrate the generality of this approach.

Results and Discussion

Synthesis and NO release characteristics of anionic piperazine diazeniumdiolates. *N*-Substituted piperazines are easily prepared, with many being commercially available. We have reacted a selection of these (**5**) with nitric oxide gas at 4-6 atmospheres of pressure in methanolic sodium methoxide, as shown in Scheme 1. The N^4-substituent of **6** may be a base labile protective group as in **6a** or **b**, or it can be acid sensitive such as that in **6c**. Commercially available *N*-aryl piperazines **5d** and **e** proved similarly amenable to diazeniumdiolation.

While we at first envisioned these products merely as one step en route to our proposed linker reagents, they proved to be useful NO-releasing agents themselves. To address the need for a fluorescent diazeniumdiolate for use in biological experiments, for example, **5f** was synthesized and converted to the diazeniumdiolate **6f**. This dansyl derivative has shown useful pharmacological properties beyond its fluorescence. Local infusion of this compound ("GLO/NO") at the luminal surface of mechanically injured porcine carotid arteries led to significant reductions of platelet adhesion at the injury site, together with marked improvement in blood flow 24 h after injury relative to control animals receiving no drug.[14] In view of this utility, we determined the NO-release characteristics of each of the anionic materials **6a-f**. All dissociated in physiological buffer with first-order kinetics with half-lives of 2-6 min to generate approximately two moles of NO per mole of dissociating **6**. These data are summarized in Table 1.

Preparation of O^2-substituted piperazine diazeniumdiolates. Alkylation of diazeniumdiolates **6a-c** at their terminal oxygen, followed by removal of the protecting group at

N^4 to free a secondary amino nitrogen on the piperazine ring for subsequent reaction with electrophilic centers, can provide molecules having useful pharmacological properties. To illustrate this approach, we have carried **6a** through the transformations shown in Scheme 2. The first step was an alkylation[2] to attach a methyl group to the terminal oxygen of the diazeniumdiolate function to produce **7**. Removal of the ethoxycarbonyl group at N^4 by basic hydrolysis gave **8**, a useful intermediate that was reacted with various electrophiles to produce agents of structure **9**.

Having noted the utility of incorporating NO-releasing functions into non-steroidal antiinflammatory drugs (NSAIDs), a strategy that has in many cases allowed introduction of potent antiinflammatories devoid of the gastric toxicity often seen in many of these drugs,[15] we linked **8** to ibuprofen's carboxyl group in a condensation reaction to form amide **9a**. If the methyl group proves to be metabolically labile, such that NO is generated at or near sensitive tissues in need of protection, **9a** could be an important addition to the list of NO-releasing NSAIDs.

Similarly, incorporation of the diazeniumdiolate group into nicotinamide (vitamin B_3) was accomplished by aroylating **8** with nicotinoyl chloride to form **9b**. To demonstrate that a piperazine containing the O^2-methyl diazeniumdiolate group can be linked to an amino acid, **9c** was synthesized.

Up to this point we have discussed the introduction of the diazeniumdiolate function into a variety of other molecules by taking advantage of the nucleophilic character of the free secondary amine in the piperazine ring, in particular by linking it via amide formation with carboxyl groups of bioactive molecules. The same nucleophilic nitrogen can combine with a reagent such as *N,N'*-disuccinimidyl carbonate to give an activated carbamate,[16] **9d**, thus transforming the

diazeniumdiolated piperazine into an electrophile. Carbamate and urea functions may now be established at the distal nitrogen of the compound containing a diazeniumdiolate. Nucleophilic termini (N, O or S) of amino acids, oligopeptides, proteins, or synthetic polymers may now be used to incorporate diazeniumdiolated piperazine moieties. For example, coupling of **9d** with N^{α}-acetyllysine methyl ester, a reaction in which the nucleophilic ϵ-nitrogen displaces the hydroxysuccinimide residue, formed the derivative **9e**.

Polyethylene glycol (PEG) has been used for many years as a covalent modifier of a variety of substrates in order to make them suitable for biological applications.[17] Attachment of a diazeniumdiolated piperazine to a PEG derivative may be useful for altering the solubility and pharmacokinetics[18] of the NO donor. To demonstrate the feasibility of this type of linkage, low molecular weight poly(ethylene glycol) methyl ether-350 was reacted with electrophile **9d** in the presence of potassium t-butoxide to give oligomer-bound diazeniumdiolate **9f**.

To illustrate the preparation of a diazeniumdiolated nucleoside, **8** was reacted with 6-chloropurine riboside to produce **9g** in an adaptation of the procedure of Iwamura et al.[19] This strategy could serve as an approach to diazeniumdiolation of oligonucleotides and nucleic acids.

Like the methyl group, an O^2-vinyl substituent can often be oxidatively removed by enzymes found in certain organs, as was demonstrated through the introduction of liver-selective NO donor **4**.[10] To extend this concept to other molecules with functionality of pharmacological relevance, we have synthesized **12** via the route shown in Scheme 3. Introduction of the vinyl group was accomplished efficiently with the aid of a novel bromoethylating agent, $BrCH_2CH_2OSO_2Cl$. Compound **12** can be used to incorporate O^2-vinylated diazeniumdiolate

moieties into other molecules in the same way that **8** was employed in synthesizing O^2-methyl derivatives **9** (Scheme 2).

In contrast to the methyl derivatives shown in Scheme 2, introduction of O^2-methoxymethyl substituents on the terminal oxygen as acetals of the diazeniumdiolate function should allow for two-stage hydrolytic release of NO as well as for potential metabolic removal. The preparations of six such O^2-methoxymethyl derivatives are shown in Scheme 4. To prepare phospholipid **13d**, phosphatidyl ethanolamine was reacted with intermediate **13c** to form a urea linkage. The immediate precursor to **13c**, **13b**, was also reacted with ethylene sulfide to produce **13e**, an agent used to couple the diazeniumdiolate function to a protein in a previously reported preliminary experiment.[20] To determine whether an NO-releasing poly(vinyl chloride) (PVC) derivative could be prepared, solutions of PVC in tetrahydrofuran were refluxed with **13b** to produce diazeniumdiolated PVC of structure **13f**. The product's elemental analytical data and NMR spectrum indicated that 1-2% of the chloride in the polymer had been replaced by **13b**. This material, when blended with various combinations of plasticizers and ionophores, has proven useful as a thromboresistant coating for intravascular biosensors and other surfaces in contact with blood.[21]

NO release characteristics of O^2-substituted piperazine diazeniumdiolates. The prediction that an O^2-methoxymethylated diazeniumdiolate can serve as a slow-release source of molecular NO was confirmed by chemiluminescence analysis of the gaseous product swept periodically from the incubation mixture after dissolution of **13b** at a concentration of 20-50 mM in 0.1 M phosphate buffer (pH 7.4) at 37 °C. Production of molecular NO proceeded rather smoothly for several weeks. Rate data for three separate determinations are presented in Figure

1. Graphical analysis of these semilog plots as previously described[9] indicated that complete dissociation of **13b** generated 1.06 ± 0.59 mol of NO per mol of **13b** that was present at time zero. In addition, *N*-nitrosopiperazine was detected when the reaction mixture was examined by HPLC after most of the NO had been released; the mechanistic origin of this product is currently under investigation. The half-life for NO release by **13b** in pH 7.4 buffer at 37 °C was estimated from the data of Figure 1 to be 17.2 ± 1.5 days [$k = (4.67 \pm 0.43) \times 10^{-7}$ s^{-1}].

A slow release of NO extending over several weeks was also observed with phospholipid derivative **13d**, as shown in Figure 2. It was noted that **13d** was incompletely soluble at the concentration used (5 mg/mL). Despite this fact, the two-phase mixture provided a remarkably steady rate of NO generation over the first 3 weeks of incubation. Integration of the data for Figure 2 indicated that 3.1 ± 1.2 mol of NO was generated for each mol of **13d** initially present. The half-life was estimated graphically to be 11.4 days.

In contrast to the spontaneous dissociation of the O^2-methoxymethyl diazeniumdiolates, the O^2-methyl and -vinyl derivatives, **8** and **12**, respectively, produced no observable chemiluminescence response at a detection limit of 12 pmol of NO when solutions thereof were incubated under the conditions used for **13b**.

Conclusion

The delivery of NO to a specific organ or cell type where it is needed without affecting other parts of the anatomy is a crucial goal in our research. We have successfully utilized the chemistry of the dibasic amine piperazine to introduce the diazeniumdiolate moiety, [N(O)NO$^-$],

into a variety of substrates. The chemistry described in this paper serves as a model for converting natural products, drugs, and other important materials to NO-releasing form.

Experimental Section

NO was purchased from Matheson Gas Products (Montgomeryville, PA). Ultraviolet (UV) spectra were recorded (in water except as otherwise indicated) on a Hewlett-Packard Model 8451A diode array spectrophotometer. Nuclear magnetic resonance (NMR) spectra were collected with a 300-MHz Varian Unity Plus or a Varian XL-200 NMR spectrometer in deuteriochloroform (unless otherwise specified). Chemiluminescence measurements of nitric oxide were performed with a Thermal Energy Analyzer Model 502A or 610 (Thermedics, Inc., Woburn, MA). Elemental analyses were done by Atlantic Microlab, Inc. (Norcross, GA). Sulfuryl chloride and piperazine derivatives were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Sodium 1-(4-Ethoxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 6a. A solution of 20 g (0.126 mol) of 1-ethoxycarbonylpiperazine 5a in 60 mL of methanol was placed in a Parr bottle. The solution was treated with 27.4 mL (0.126 mol) of 25% sodium methoxide in methanol. The system was evacuated, charged with 40 psi of nitric oxide and kept at 25 °C for 48 h. The white crystalline product was collected by filtration and washed with cold methanol as well as with copious amounts of ether. The product was dried under vacuum to give a 14.5 g (48%) yield of **6a**: mp 184-185 °C; UV (0.01 M NaOH) λ_{max} (ε) 252 nm (10.4 mM^{-1} cm^{-1}); NMR (0.1 M sodium deuteroxide) δ 1.25 (t, 3 H), 3.11 (m, 2 H), 3.68 (m, 2 H), 4.15 (q, 2 H).

Anal. Calcd for $C_7H_{13}N_4O_4Na$: C, 35.00; H, 5.46; N, 23.33; Na, 9.57. Found: C, 34.87; H, 5.53; N, 23.26; Na, 9.69.

Sodium 1-(4-Benzyloxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 6b. 1-Benzyloxycarbonylpiperazine (23.5 g, 107 mmol) in 6 mL (120 mmol) of 25% methanolic sodium methoxide and 60 mL of methanol was exposed to NO as described for the preparation of **6a**: mp 180 °C (dec.); UV (0.01 M NaOH) λ_{max} (ϵ) 252 nm (6.3 mM^{-1} cm^{-1}); NMR (0.1 M NaOD in D_2O) δ 3.12 (m, 4 H), 3.70 (m, 4 H), 5.18 (s, 2 H), 7.45 (s, 5 H). Anal. Calcd for $C_{12}H_{15}N_4O_4Na \cdot 0.5$ NaOH: C, 44.72; H, 4.85; N, 17.39; Na, 10.70. Found: C, 44.75; H, 4.78; N, 17.59; Na, 10.32.

Sodium 1-(4-t-Butoxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 6c. This reaction was carried out as described for the preparation of **6a**. A solution of t-butoxycarbonylpiperazine 5c (11.5 g; 62 mmol) and 15 mL (69 mmol) of 25 % methanolic sodium methoxide in 30 mL of methanol was exposed to nitric oxide to give 7 g (42%) of **6b** as a free flowing solid: mp 262-264 °C; UV (0.01 M NaOH) λ_{max} (ϵ) 252 nm (6.7 mM^{-1} cm^{-1}); NMR (0.1 M NaOD in D_2O) δ 1.47 (s, 9 H), 3.11 (m 4 H), 3.65 (m, 4 H). Anal. Calcd for $C_9H_{17}N_4O_4Na$: C, 40.30; H, 6.39; N, 20.89; Na, 8.57. Found: C, 40.09; H, 6.44; N, 20.64; Na, 8.69.

Sodium 1-[4-(Pyrimidin-2-yl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 6d. A solution of 4 g (0.024 mmol) of 1-(2-pyrimidyl)piperazine, **5d**, in 50 mL of methanol and 10 mL of ether was placed in a Parr bottle with 5.2 mL of 25% sodium methoxide in methanol. This was exposed to nitric oxide as described above to give 1.7 g (25%) of **6d** as large white needles: mp 199-200 °C; UV (0.01 M NaOH) λ_{max} (ϵ) 246 nm (20.1 mM^{-1} cm^{-1}) and 306 nm (1.7

$mM^{-1}cm^{-1}$); NMR (DMSO-d_6) δ 2.5 (m, 4 H), 3.89 (m, 4 H), 6.66 (t, 1 H), 8.38 (d, 2 H). Anal. Calcd for $C_8H_{11}N_6O_2Na$: C, 39.03; H, 4.50; N, 34.13; Na, 9.34. Found: C, 38.94; H, 4.44; N, 34.10; Na, 9.53.

Sodium 1-(4-Phenylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 6e. Exposure of 15 g (0.093 mol) of 1-phenylpiperazine to NO as described above gave 7.23 g (33%) of **6e** as white crystals: mp 215-216 °C; UV (0.01 M NaOH) λ_{max} (ϵ) 246 nm (20.2 mM^{-1} cm^{-1}) ; NMR (0.1 M NaOD in D_2O) δ 3.37 (m, 8 H), 7.16 (m, 3 H), 7.42 (m, 2 H). Anal. Calcd for $C_{10}H_{13}N_4O_2Na$: C, 49.18; H, 5.37; N, 22.94; Na, 9.41. Found: C, 48.94; H, 5.28; N, 22.81; Na, 9.63.

Sodium 1-[4-(5-Dimethylamino-1-naphthalenesulfonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 6f. A solution containing 7.98 g (9.27 mmol) of piperazine and 5 g (18.5 mmol) of dansyl chloride in 100 mL of toluene was heated at reflux for 6 h. The product was isolated after washing the solution with 5% sodium hydroxide and then water and the organic layer was dried over sodium sulfate. The solution was filtered and concentrated on a rotary evaporator to give 5 g (84%) of dansylpiperazine, **5f**, as a yellow-green powder. To prepare the diazeniumdiolate, a solution of 3.08 g (9.64 mmol) of **5f**, 2.2 mL (9.64 mmol) of 25% sodium methoxide in methanol and 25 mL of *N,N*-dimethylformamide was exposed to 5 atmospheres of NO gas for two days. After flushing with argon, 150 mL of ether was added and 2 g (52%) of **6f** was isolated by filtration: mp 158-160 °C; UV (methanol) λ_{max} (ϵ) 252 nm (12.7 mM^{-1} cm^{-1}) and 344 nm (3.0 mM^{-1} cm^{-1}); the compound fluoresced with an emission wavelength of 523 nm (excitation wavelength of 342 nm); NMR (D_2O) δ 2.8 (s, 6 H), 3.15 (m, 4 H), 3.45 (m, 4 H), 7.5 (m, 3 H), 8.35 (m, 3 H). Anal. Calcd for $C_{16}H_{20}N_5O_4SNa$: C, 47.87; H, 5.02; N, 17.45; S, 7.99. Found: C, 47.94; H, 5.06; N, 17.32; S, 8.04.

O^2-Methyl 1-(4-Eth xycarb nylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 7. To a solution of 10 g (0.042 mol) of diolate **6a** in 200 mL of methanol was added 10 g of anhydrous potassium carbonate and the resulting slurry was cooled in an ice bath. Dimethyl sulfate (4.7 mL, 0.05 mol) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm gradually to room temperature. After an additional hour the solution was concentrated on a rotary evaporator; the residue was extracted with dichloromethane, washed with water, dried over sodium sulfate and filtered through a layer of magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel. Elution with 2:1 dichloromethane:ethyl acetate provided 5.7 (56%) of **7** as an oil, which crystallized on standing: mp 46 °C; UV λ_{max} (ε) 240 nm (8.4 mM^{-1} cm^{-1}); NMR δ 3.38 (m, 4 H), 3.67 (m, 4 H), 4.03 (s, 3 H), 4.16 (q, 2 H). Anal. Calcd for $C_8H_{16}N_4O_4$: C, 41.38; H, 6.90; N, 24.14. Found: C, 41.23; H, 6.82; N, 24.05.

O^2-Methyl 1-(Piperazin-1-yl)diazen-1-ium-1,2-diolate, 8. A mixture of 6.42 g (0.0276 mmol) of **7**, 200 mL of 10% sodium hydroxide in ethanol and 5 mL of water was heated at reflux. After 45 min no starting material remained in the mixture, as assessed from qualitative thin layer chromatography. The solution was allowed to cool to room temperature and evaporated to a viscous residue, which was extracted with dichloromethane, dried over sodium sulfate, filtered, and evaporated to give 3.8 g of a yellow oil. The product was chromatographed on silica gel using a Flash 40 system eluted with 9:2:0.1 dichloromethane:methanol:water to give 3.22 g (73%) of **7** as a pale yellow oil: mp (picrate) 113-114 °C; UV λ_{max} (ε) 234 nm (7.0 mM^{-1} cm^{-1}); NMR δ 3.03 (m, 4 H), 3.38 (m, 4 H), 4.06 (s, 3 H). Anal. Calcd for picrate salt $C_{11}H_{15}N_7O_9 \cdot 0.5$ C_2H_5OH: C, 35.00; H, 4.28; N, 23.81. Found: C, 35.04; H, 4.43; N, 23.75.

O^2-Methyl 1-[4-(p-Isobutyl-α-methylphenylacetyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 9a. A solution of 1.72 g (0.0083 mmol) of Ibuprofen in 5 mL of thionyl chloride was stirred with a boiling stone at room temperature for 15 h. Excess thionyl chloride was removed on a rotary evaporator and the residue was placed under high vacuum to remove traces of the chlorinating agent. The resulting residue was taken up in dichloromethane, washed with 5% sodium bicarbonate solution, dried over sodium sulfate, filtered and evaporated to give 1.76 g (94%) of the acid chloride as a colorless oil. Without any further purification, 1.7 g (7.5 mmol) of p-isobutyl-α-methylphenylacetyl chloride was added to a cold solution of **8** in dichloromethane containing a slight molar excess of triethylamine (1.11 mL; 8 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h, diluted with 20 mL of dichloromethane, washed with 5% aqueous hydrochloric acid followed by 10% sodium hydroxide, dried and evaporated to give 2.6 g of a yellow oil that crystallized on standing. Recrystallization from ether:petroleum ether gave an analytically pure sample: mp 79-80 °C; UV λ_{max} (ϵ) 224 nm (12.4 mM^{-1} cm^{-1}), 234 (8.7 mM^{-1} cm^{-1}); NMR δ 0.88 (d, 6 H), 1.44 (d, 3 H), 1.84 (m, 1 H), 2.45 (d, 2 H), 3.36 (m, 4 H), 3.52 (m, 4 H), 3.83 (q, 1 H), 3.97 (s, 3 H), 7.11 (s, 4 H). Anal. Calcd for $C_{18}H_{28}N_4O_4$: C, 62.05; H, 8.10; N, 16.08. Found: C, 61.80; H, 8.03; N, 16.04.

O^2-Methyl 1-[(4-Nicotinoyl)piperazin-1-yl)diazen-1-ium-1,2-diolate, 9b. To a solution of 306 mg (1.91 mmol) of **8** in 10 mL of dichloromethane was added 340 mg (1.91 mmol) of nicotinyl chloride hydrochloride. The resulting mixture was treated with 2 mL of triethylamine and stirred for 30 min, then washed with water. The organic layer was dried over sodium sulfate, filtered through a layer of magnesium sulfate and evaporated. The residue was chromatographed on silica gel using 1:1 dichloromethane:acetone as eluant. Evaporation of the

solvent gave 485 mg of a crystalline product: mp 85-86 °C; UV λ_{max} (ϵ) 236 nm (10 mM^{-1} cm^{-1}); NMR δ 3.47 (m, 4 H), 3.83 (m, 4 H), 4.04 (s, 3 H), 7.29 (m, 2 H), 7.82 (m, 1 H), 8.72 (m, 1 H). Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.66; N, 26.42. Found: C, 50.01; H, 5.76; N 26.24.

O^2-Methyl 1-[4-(*N*-Acetyl-L-methionyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 9c. To a solution of 1.18 g (0.0062 mol) of *N*-acetyl-L-methionine in 20 mL of 2:1 dichloromethane:acetonitrile was added a solution 1.44 g (0.007 mol) of dicyclohexylcarbodiimide (DCC) in 10 mL of dichloromethane. This was followed by the rapid introduction of 805 mg (0.007 mol) of *N*-hydroxysuccinimide in 6 mL of dichloromethane. The resulting mixture was stirred at room temperature for 5 min then 1 g (0.0062 mmol) of **8** in 10 mL of dichloromethane was added dropwise. The reaction mixture was stirred at 25 °C for 4 h. A few drops of glacial acetic acid were added to decompose excess DCC. The mixture was filtered and washed with dilute hydrochloric acid followed by dilute aqueous sodium hydroxide. The organic layer was dried over sodium sulfate, filtered through a layer of magnesium sulfate, and evaporated in vacuo to give 2 g of a yellow oil. Purification of the product was accomplished on a Flash 40 system using a 4 x 15-cm KP-Sil column eluted with 10:10:1 dichloromethane:ethyl acetate:methanol to give 785 mg (38%) of a pale yellow oil: UV λ_{max} (ϵ) 230 nm (8.7 mM^{-1} cm^{-1}); NMR δ 2.02 (s, 3 H), 2.07 (m, 2 H), 2.11 (s, 3 H), 3.34 (s, 1.5 H), 3.46 (m, 4 H), 3.83 (m, 4 H), 4.03 (s, 3 H), 5.15 (m, 1 H), 6.28 (b, 0.5 H), 6.35 (b, 0.5 H). Anal. Calcd for $C_{12}H_{23}N_5O_4S$ • 0.5 CH_3OH: C, 42.97; H, 7.21; N, 20.04; S, 9.18. Found: C, 43.30; H, 6.91; N, 20.08; S, 9.40.

O^2-Methyl 1-[4-(Succinimid-*N*-oxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 9d. A partial solution of 2.8 g (0.011 mol) of *N,N'*-disuccinimidyl carbonate in 15 mL of dichloromethane was stirred in a round-bottom flask and mixed with a solution of 1.76 g (0.011 mol) of **8** in 30 mL of dichloromethane and 1.5 mL of triethylamine. The resulting homogeneous solution was stirred at room temperature. The progress of the resulting reaction was monitored on silica gel thin layer chromatography using 1:1 dichloromethane:ethyl acetate. After stirring for 1 h, the solution was washed with 1 M hydrochloric acid, followed by 5% aqueous sodium hydroxide. The solution was dried as described previously and evaporated under vacuum to give 2.44 g of a glassy substance. Purification of the product was carried out on a Biotage Flash 40 system with a 4.0 x 15.0 cm KP-Sil column. The system was eluted with 1:1 dichloromethane:ethyl acetate at 15 psi of air at a rate of elution of 25 mL/min to give pure **9d**: mp 104-105 °C; UV λ_{max} (ϵ) 234 nm (6.44 mM^{-1} cm^{-1}); NMR δ 2.85 (s, 4 H), 3.52 (m, 4 H), 3.74 (b, 2 H), 3.82 (b, 2 H), 4.04 (s, 3 H). Anal. Calcd for $C_{10}H_{15}N_5O_6$: C, 39.87; H, 5.02; N, 23.25. Found: C, 39.92; H, 4.97; N, 23.07.

Reaction of 9d with N^{α}-Acetyl Lysine Methyl Ester to Form 9e. To a solution of 197 mg (0.654 mmol) of **9d** in 10 mL of chloroform was added 167 mg (0.7 mmol) of N^{α}-acetyl lysine methyl ester hydrochloride followed by 278 μL (2 mmol) of triethylamine. The resulting solution was heated at reflux for 8 h, allowed to cool to room temperature and washed with water. The organic layer was dried over sodium sulfate, filtered and evaporated to give 160 mg of a yellow glass. Purification was carried out on a Flash 40 apparatus using a 7.5 x 4 cm KP-Sil column and eluted with 10:1 dichloromethane:methanol to give 39 mg of pure **9e**: UV (ethanol) λ_{max} 240 nm (8.2 mM^{-1} cm^{-1}); NMR δ 1.36 (m, 2 H), 1.55 (m, 2 H), 1.81 (m, 2 H), 2.04 (s, 3 H), 3.22 (m, 2

H), 3.40 (m, 4 H), 3.57 (m, 4 H), 3.75 (s, 3 H), 4.03 (s, 3 H), 5.0 (b, 1 H), 6.42 (d, 1 H). Anal. Calcd for $C_{15}H_{28}N_6O_6 \cdot H_2O$: C, 44.33; H, 7.44; N, 20.68. Found: C, 44.40; H, 7.12; N, 20.18.

Reaction of 9d with Poly(ethylene Glycol) Methyl Ether to Form 9f. A partial solution of 700 mg (2.0 mmol) of poly(ethylene glycol) methyl ether (average MW 350) and 246 mg (2.2 mmol) of potassium t-butoxide in 5 mL of tetrahydrofuran and 1 mL of t-butanol was heated at reflux for 30 min. To the hot solution was added 700 mg (2.33 mmol) of **9d** in 15 mL of tetrahydrofuran and heating at reflux was continued for 3 h. The reaction mixture was allowed to cool to room temperature, diluted with 50 mL of dichloromethane and washed with 5% aqueous hydrochloric acid. The solution was dried over sodium sulfate, filtered and evaporated to give 716 mg of **9f** as a yellow oil. This material was chromatographed on a Flash 40 system using a 4.0 x 15 cm KP-Sil column and 4:1 acetonitrile:tetrahydrofuran: UV λ_{max} (ϵ) 238 nm (6.4 mM^{-1} cm^{-1}); NMR δ 3.38 (s, 3 H), 3.41 (m, 4 H), 3.56 (m, 7 H), 3.65 (m, 25 H), 4.03 (s, 3 H). Anal. Calcd for $C_{21}H_{42}N_4O_{11}$: C, 49.06; H, 7.86; N, 10.40. Found: C, 48.87; H, 7.96; N, 10.83.

***O*²-Methyl 1-{4-[7-(β-Ribofuranosyl)purin-6-yl]piperazin-1-yl}diazen-1-ium-1,2-diolate, 9g.** To a solution of 275 mg (1.72 mmol) of **8** in 10 mL of ethanol and 0.250 mL (1.8 mmol) of triethylamine was added 460 mg (1.6 mmol) of 6-chloropurine riboside. The resulting slurry was heated at reflux for a total of 3 h. A homogeneous solution resulted upon heating. The solution was allowed to cool to room temperature and concentrated on a rotary evaporator to give a solid residue. The product was extracted with acetone, filtered and evaporated to give 689 mg of a white powder then recrystallized from ethanol: mp 195-197 °C; UV λ_{max} (ϵ) 218 nm (15.1 mM^{-1} cm^{-1}), 278 (17.2 mM^{-1} cm^{-1}); NMR (DMSO-d_6) δ 3.93 (s, 3 H), 4.1-4.58 (m, 8 H),

5.23 (m, 1 H), 5.32 (m, 1 H), 5.50 (d, 1 H), 5.93 (d, 1 H), 8.30 (s, 1 H), 8.48 (s, 1 H). Anal. Calcd for $C_{15}H_{22}N_8O_6$: C, 43.90; H, 5.40; N, 27.30. Found: C, 43.78; H 5.38; N, 27.29.

2-Bromoethylsulfuryl Chloride. A solution of 20 mL (0.28 mol) of bromoethanol in 50 mL of dichloromethane was cooled to 0 °C, followed by the dropwise addition of 11.25 mL (0.28 mol) of sulfuryl chloride in 50 mL of dichloromethane. The resulting solution was kept at 4 °C for 72 h. The solution was washed with cold 10% sodium hydroxide until the washings tested distinctly basic. The organic layer was dried over sodium sulfate, filtered through a layer of magnesium sulfate, and concentrated on a rotary evaporator; the resulting crude product was vacuum distilled to give 35 g (56%) of a colorless oil: bp 73-75 °C at 1.5 mmHg; NMR, δ 3.64 (t, 2 H), 4.75 (t, 2 H). Anal. Calcd for $C_2H_4SO_3ClBr$: C, 10.75; H, 1.80; S, 14.35; total halogen as Br, 71.52 and Cl, 31.72. Found: C, 10.82; H, 1.80; S, 14.35; total halogen as Br 71.63 and as Cl, 31.78.

***O*²-(2-Bromoethyl) 1-(4-Ethoxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 10a.** To a cold (0 °C) slurry of 4.0 g (16.7 mmol) of **6a** and 4 g of anhydrous sodium carbonate in 60 mL of methanol was added 3.75 g (16.8 mmol) of 2-bromoethyl sulfuryl chloride in 5 mL of ether under nitrogen. The reaction mixture was allowed to warm to 10 °C and stirred for 2 h. The mixture was filtered, the filtrate was concentrated on a rotary evaporator and the residue was extracted with dichloromethane. The solution was filtered through a layer of magnesium sulfate and evaporated in vacuo to give a tan solid that on recrystallization from petroleum ether:ether gave 540 mg (10%) of pale yellow plates: mp 83-84 °C; UV λ_{max} (ϵ) 240 nm (7.4 mM^{-1} cm^{-1}); NMR (methanol-d_4) δ 1.28 (t, 3 H), 3.41 (m, 4 H), 3.57 (t, 2 H), 3.67 (m, 4 H), 4.16 (q, 2 H),

4.49 (t, 2 H). Anal. Calcd for $C_9H_{17}N_4O_4Br$: C, 33.24; H, 5.27; N, 17.23; Br, 24.57. Found: C, 33.40; H, 5.19; N, 17.23; Br, 24.68.

***O*²-(2-Bromoethyl) 1-(4-t-Butoxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 10b.** A slurry of 6.9 g (26 mmol) of **6b**, 25 g of anhydrous sodium carbonate and 200 mL of methanol was cooled to 0 °C. A solution of 6 g (27 mmol) of 2-bromoethyl sulfuryl chloride in 10 mL of ether was added dropwise to the cold slurry under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred overnight to give on workup 3.9 g of a brown solid. The crude product was recrystallized from ether:petroleum ether to give 1 g of a white crystalline diazeniumdiolate: mp 86-87 °C; UV (methanol) λ_{max} (ϵ) 236 nm (8.9 mM^{-1} cm^{-1}); NMR δ 1.47 (t, 9 H), 3.34 (m, 4 H), 3.59 (m, 6 H), 4.49 (t, 2 H). Anal. Calcd for $C_{11}H_{21}N_4O_4Br$: C, 37.41; H, 5.99; N, 15.86; Br, 22.62. Found: C, 37.49; H, 6.03; N, 15.87; Br, 22.65.

***O*²-Vinyl 1-(4-Ethoxycarbonylpiperazin-1-yl)diazen-1-ium-1,2-diolate, 11a.** To a solution of 917 mg (2.8 mmol) of **10a** in 55 mL of tetrahydrofuran was added 1 g (25 mmol) of powdered sodium hydroxide. The resulting slurry was heated at reflux for 3 h, allowed to cool to room temperature and filtered. The solvent was evaporated and the residue was extracted with dichloromethane, dried over sodium sulfate, filtered through magnesium sulfate and concentrated under vacuum to give 280 mg of an orange oil. This was purified on a silica gel column with 1:1 ethyl acetate:cyclohexane as the eluant to give 268 mg (39%) of **11a**: UV (methanol) λ_{max} (ϵ) 260 nm (6.6 mM^{-1} cm^{-1}); NMR δ 1.28 (t, 3 H), 3.47 (m, 4 H), 3.68 (m, 4 H), 4.17 (q, 2 H), 4.44 (dd, 1 H), 4.869 (dd, 1 H), 6.82 (dd, 1 H). Anal. Calcd for $C_9H_{16}N_4O_4$: C, 44.26; H, 6.60; N, 22.94. Found: C, 44.18; H, 6.53; N, 22.87.

***O*²-Vinyl 1-(Piperazin-1-yl)diazen-1-ium-1,2-diolate, 12.** A solution of 400 mg (1.64 mmol) of **11a** in 6 mL of 9% sodium hydroxide in ethanol and 2 mL of water was heated at reflux for 4 h. The reaction mixture was allowed to cool to room temperature and concentrated under vacuum. The residue was extracted with dichloromethane. The organic extract was in turn extracted with cold 1 M hydrochloric acid and the organic layer was discarded. The aqueous layer was washed once with dichloromethane, basified with 10% aqueous sodium hydroxide and extracted with dichloromethane. This organic extract was dried over sodium sulfate, filtered through magnesium sulfate and concentrated under vacuum to give a pale yellow oil. The crude product was chromatographed on silica gel with 20:1 acetonitrile:tetrahydrofuran as the eluant to give 200 mg of **12**: mp 133-135 °C (picrate); UV (methanol) λ_{max} (ε) 260 nm (8.7 mM^{-1} cm^{-1}); NMR δ 3.05 (m, 4 H), 3.46 (m, 4 H), 4.11 (dd, 1 H), 4.86 (dd, 1 H), 6.82 (dd, 1 H); maximum solubility in phosphate-buffered saline, 76 mM. Anal. Calcd for picrate $C_{12}H_{15}N_7O_9$: C, 35.92; H, 3.77; N, 24.43. Found: C, 35.74; H, 3.73; N, 24.30.

***O*²-(Methoxymethyl) 1-[(4-Ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 13a.** A slurry of 4.4 g (0.018 mol) of **6a** and 2 g of anhydrous sodium carbonate in 100 mL of tetrahydrofuran was stirred under nitrogen at 0 °C. Through a septum was added 1.5 mL (0.02 mmol) of methyl chloromethyl ether dropwise. Methanol, 1 mL, was added simultaneously with a syringe. The resulting mixture was allowed to warm up to room temperature and stirred overnight. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue was extracted with dichloromethane, dried over sodium sulfate, filtered through a layer of magnesium sulfate and evaporated to give 4.74 g of an oil which crystallized after several days on standing at ambient temperature. The product was recrystallized from ether to give pale

yellow plates: mp 54-55 °C; UV λ_{max} (ϵ) 232 nm (7.3 mM^{-1} cm^{-1}); NMR δ 1.28 (t, 3 H), 3.46 (m, 4 H), 3.46 (s, 3 H), 3.65 (m, 4 H), 4.16 (q, 2 H), 5.22 (s, 2 H). Anal. Calcd for $C_9H_{18}N_4O_5$: C, 41.22; H, 6.87; N, 21.37. Found: C, 41.32; H, 6.95; N, 21.42.

***O*²-Methoxymethyl 1-(Piperazin-1-yl)diazen-1-ium-1,2-diolate, 13b.** A solution of 3.51 g (13.4 mol) of **13a** in 100 mL of 10% ethanolic sodium hydroxide was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and the ethanol was removed on a rotary evaporator. The residue was extracted with dichloromethane, filtered, and extracted with aqueous hydrochloric acid. The aqueous layer was washed with dichloromethane then made basic with aqueous sodium hydroxide. The product was extracted into dichloromethane, dried over sodium sulfate and filtered through a layer of magnesium sulfate. Evaporation of the solvent gave 2.1 g of product as a pale yellow oil. Purification was carried out by Flash 40 chromatography using a 4 x 15 cm KP-Sil column and 10:1 dichloromethane:methanol as the eluent: UV λ_{max} (ϵ) 232 nm (8.2 mM^{-1} cm^{-1}); NMR δ 3.05 (m, 4 H), 3.46 (m, 4 H), 3.47 (s, 3 H), 5.22 (s, 2 H); maximum solubility in phosphate-buffered saline, 0.36 M. Anal. Calcd for picrate $C_{12}H_{17}N_7O_{10}$: C, 34.37; H, 4.09; N, 23.38. Found: C, 34.63; H, 4.13; N, 23.13.

***O*²-Methoxymethyl 1-[4-(Succinimid-*N*-oxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 13c.** A partial solution of 1.23 g (4.8 mmol) of *N,N'*-disuccinimidyl carbonate in 15 mL of dichloromethane was added to a solution of 915 mg (4.8 mmol) of **13b** in 15 mL of dichloromethane. To the resulting homogeneous solution was added 0.7 mL (5 mmol) of triethylamine. The reaction was complete on stirring at room temperature within 1 h. The reaction mixture was evaporated to dryness. The residue was taken up in dichloromethane and washed first with cold, dilute hydrochloric acid then with aqueous bicarbonate. The organic layer

was dried over sodium sulfate, filtered through magnesium sulfate and evaporated to give 1.42 g of a glass. This compound was purified as described for the methyl analogue **9d** giving a glass that crystallized on standing: mp 123-125 °C; UV (ethanol) λ_{max} (ϵ) 238 nm (7.69 mM^{-1} cm^{-1}); NMR δ 2.82 (s, 4 H), 3.49 (s, 3 H), 3.57 (m, 4 H), 3.81 (m, 4 H), 5.22 (s, 2 H). Anal. Calcd for $C_{11}H_{17}N_5O_7$: C, 39.88; H, 5.17; N, 21.14. Found: C, 40.16; H, 5.23; N, 20.91.

Conjugation of 13b with a Phospholipid to Produce 13d. To a solution of 170 mg (0.513 mmol) of **13c** in 10 mL of chloroform was added 200 μL (1.4 mmol) of triethylamine and 346 mg (0.5 mmol) of dipalmitoyl DL-α-phosphatidyl ethanolamine. The partial solution was diluted with 10 mL of chloroform to give a homogeneous solution. Heating was continued for 8 h, whereupon the mixture was allowed to cool to room temperature and concentrated on a rotary evaporator to give 730 mg of a solid residue. Purification of **13b** was performed on a Flash 40 system using a 4 x 15 cm KP-Sil column. The less polar impurities were eluted with 10:1 dichloromethane:methanol while the remainder eluted in 1:1 dichloromethane:methanol to give 308 mg of product: mp 123-125 °C; UV (ethanol) λ_{max} (ϵ) 234 nm (8.4 mM^{-1} cm^{-1}); NMR δ 0.89 (t, 6 H) , 1.27 (s, 52 H), 1.58 (b, 8 H), 3.47 (m, 4 H), 3.60 (m, 4 H), 3.50 (s, 3 H), 3.93 (b, 4 H), 4.35 (m, 1 H), 5.23 (s, 2 H), 6.38 (b, 1 H). Anal. Calcd for $C_{44}H_{86}N_5O_{12}P \cdot 2H_2O$: C, 55.97; H, 9.61; N, 7.42. Found: C, 56.33; H, 9.38; N, 6.89.

***O*²-Methoxymethyl 1-[4-(2-Mercaptoethyl)piperazin-1-yl]diazen-1-ium-1,2-diolate, 13e.** A solution of 3.34 g (0.0176 mol) of **13b** in 20 mL of dichloromethane and 5 mL of methanol was treated with 6 g (0.1 mol) of ethylene sulfide. The resulting solution was heated at reflux, under nitrogen, and the progress of the reaction was monitored by thin layer chromatography (silica gel, 4:1 acetonitrile:tetrahydrofuran). The reaction appeared to have gone

to completion within 18 h; at this time the mixture was cooled to room temperature, filtered, and evaporated to give 4.2 g of a turbid yellow oil. The oil was chromatographed twice on a silica gel column and eluted with 4:1 acetonitrile:tetrahydrofuran to give 1.3 g of **13e**: mp (picrate) 45 °C; UV λ_{max} (ϵ) 232 nm (4.9 mM^{-1} cm^{-1}); NMR δ 2.66-2.85 (m, 8 H), 3.49 (s, 3 H), 3.51 (m, 4 H), 5.22 (s, 2 H). Anal. Calcd for the picrate $C_{14}H_{21}N_7O_{10}S$: C, 35.07; H, 4.42; N, 20.45; S, 6.69. Found: C, 35.23; H, 4.35; N, 20.16; S, 6.88.

Reaction of 13b with Poly(vinyl Chloride) to Produce 13f. Poly(vinyl chloride) (2.15 g, average molecular weight 62 kDa, Aldrich) was dissolved in 50 mL of tetrahydrofuran with the aid of a vortex mixer. To this solution was added 197 mg (1.04 mmol) of **13b** in 5 mL of tetrahydrofuran. The resulting solution was refluxed for 6 h then cooled to room temperature. The solvent was removed with a rotary evaporator to give an amorphous solid that was washed with methanol then water to remove unreacted **13b**. The polymer was dried under vacuum to give 2.6 g of product: NMR (tetrahydrofuran-d_8) δ 2.02-2.49 (m, 2 H), 2.93-3.01 (m, 0.03 H), 3.36-3.41 (m , 0.03 H), 3.38 (s, 0.023 H), 4.28-4.70 (m, 1 H), 5.10 (s, 0.015 H). Integration of the methylene and methyl singlets at δ 5.10 and 3.32, respectively, relative to the methylene and methinyl protons of the polymer backbone led to an estimate of 1.3 mol percent incorporation of **13b** into the poly(vinyl chloride). Elemental analysis pointed to a slightly higher incorporation, however: Anal. (Calcd on the assumption that 2.3% of the Cl in the polymer is replaced by **13b** and that tetrahydrofuran remains trapped in the polymer matrix to the extent of 0.05 solvent molecules per monomer unit): C, 40.29; H, 5.34; Cl, 49.88; N, 1.81. Found: C, 40.51; H, 5.46; Cl, 50.27; N, 1.83.

Measurement of Dissociation Kinetics and Yields of NO. Rate measurements for piperazine derivative **6a-f** were carried out spectrophotometrically by following the decrease in the UV absorption maxima in 0.10 M phosphate buffer at pH 7.4. All measurements were made using a Hewlett Packard 8451 UV-visible diode array spectrophotometer at 37 °C. Dissociation of **6a-c** was followed at the characteristic 252-nm diazeniumdiolate maximum. With **6d** and **e**, which displayed more intense UV absorption and had maxima at 246 nm, reactions were followed at that wavelength. Compound **6f** was solubilized for the kinetic runs by dissolving in methanol at a concentration of 10 mM then diluting 100-fold with pH 7.4 phosphate buffer to initiate the reaction.

For all except the pyrimidine substituted derivative, absorbance-time (A-t) data followed good first-order kinetics with $\ln(A_t-A_\infty)$ versus time plots, displaying good linearity over 3-4 half-lives. With the pyrimidine derivative, an initial drop in absorbance from 2.23 to ca 1.355 over the first 600 s was followed by a slower decrease over longer reaction times ($t > 1900$ s). Using the 1.355 absorbance value as A_∞, the initial data also showed good first-order behavior ($R^2 = 0.994$ over 3.5 half-lives).

The rates for O^2-substituted derivatives **13b** and **13d** were measured by a chemiluminescence method developed previously.[11] Briefly, solutions (or a suspension, in the case of incompletely soluble **13d**) in 0.1 M phosphate, pH 7.4, were incubated continuously at 37 °C except for short intervals during which their contents were swept with inert gas into a chemiluminescence detector for quantification of NO. After a steady baseline was achieved, integration over several minutes provided the NO generation rate during that interval. Plots of

these rates versus the times at the midpoints of the corresponding interval yielded a curve from which the half-life could be estimated.

Yields of NO were determined by integration of these rate versus time plots with extrapolation to infinite time, as previously described.[9] For anionic diazeniumdiolates **6a-f**, infinite time was effectively reached within a single purge with inert gas, allowing quantification by integration of the resulting chemiluminescence detector response versus time curve.

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Supporting Information Available: Individual least squares plots (assuming first order kinetics) of the three separate sets of NO release data found in Figure 1 (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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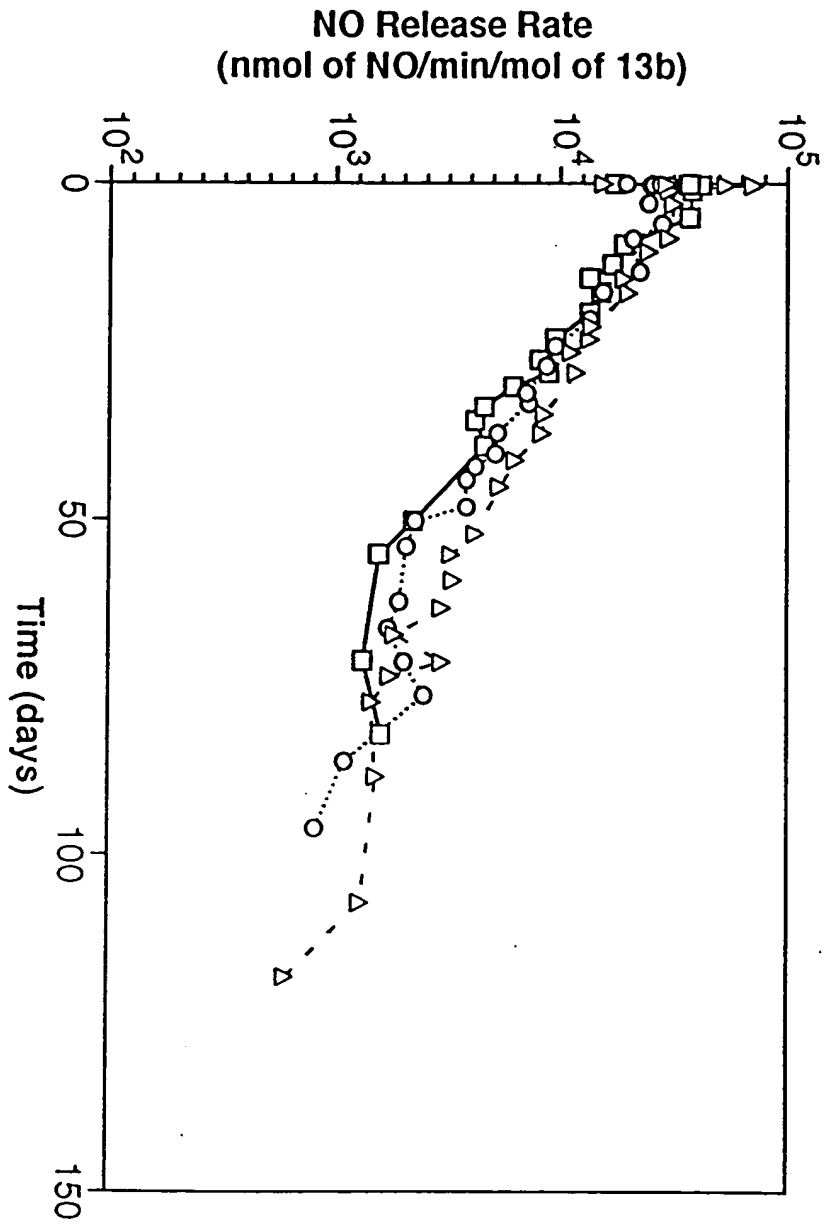
Table 1. NO Release Rates of Anionic Piperazine Diazeniumdiolates (**6**).

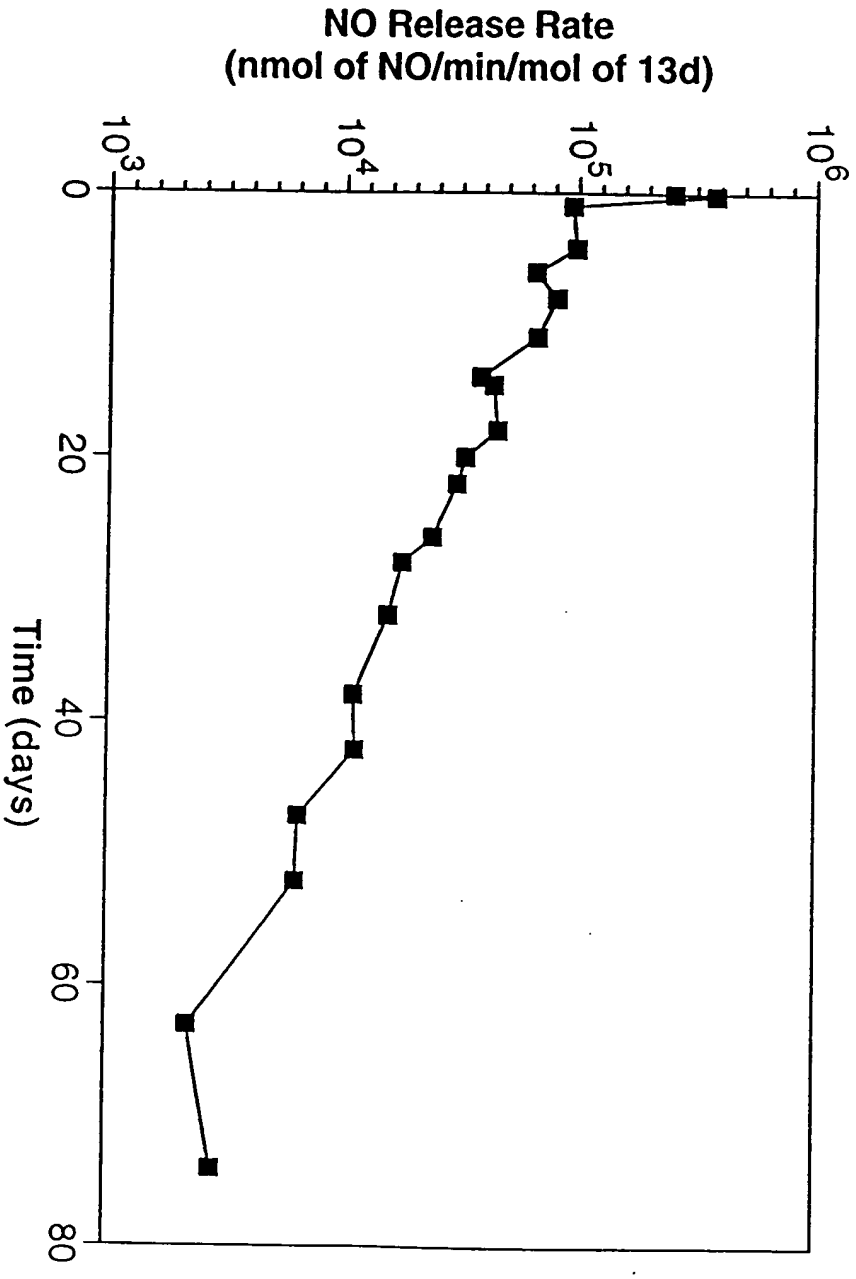
	Dissociation kinetics in pH 7.4 phosphate at 37 °C		
Compound	k (s^{-1})	Half-life (min)	Observed NO release per mole of diazeniumdiolate (mol)
6a	4.08×10^{-3}	2.8	1.92 ± 0.11
6b	5.69×10^{-3}	2.0	1.83 ± 0.06
6c	4.39×10^{-3}	2.6	1.85 ± 0.05
6d	6.14×10^{-3}	1.9	1.96 ± 0.02
6e	5.74×10^{-3}	2.0	2.36 ± 0.08
6f	2.05×10^{-3}	5.6	1.43 ± 0.17

Figure Legends

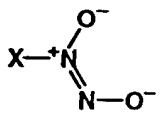
Figure 1. Observed rates of NO generation from **13b** at initial concentrations of 20-50 mM at various times after dissolution in 0.1 M phosphate buffer at 37 °C. Individual results for three separate incubations are shown. The pH remained at 7.4 throughout the incubations.

Figure 2. Time course of NO release observed on suspending 10 mg of diazeniumdiolated phospholipid **13d** in 2 mL of 0.1 M phosphate buffer (pH 7.4) at 37 °C.


NO Release Rate
(nmol of NO/min/mol of 13b)
10^2
10^3
10^4
10^5
0
50
100
150
Time (days)


NO Release Rate
(nmol of NO/min/mol of 13d)
10^3
10^4
10^5
10^6
0
20
40
60
80
Time (days)

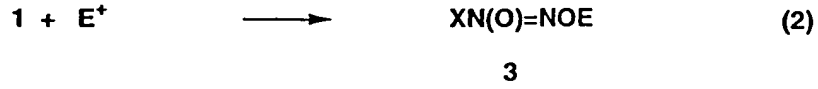

1 + H+ 2NO + XH (1)



1

$R^1N[N(O)NO]^-(CH_2)_XNH_2^+R^2$

2



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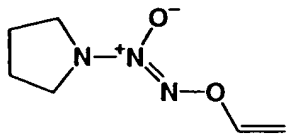

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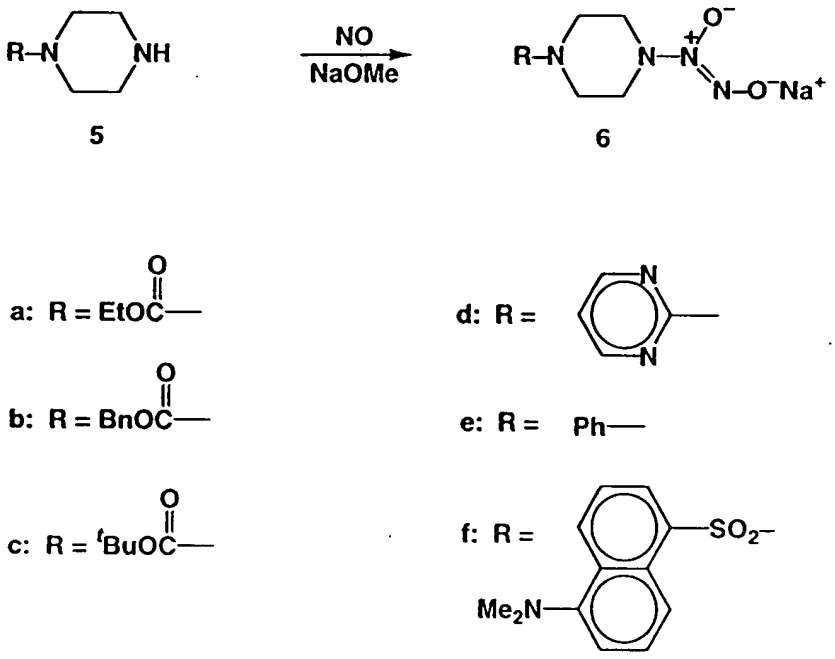
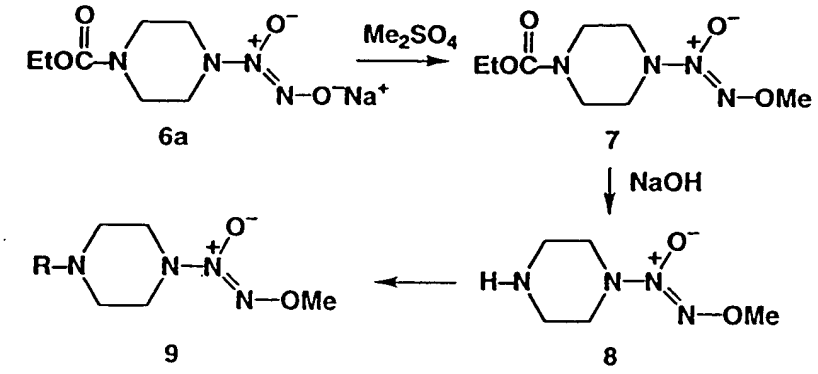
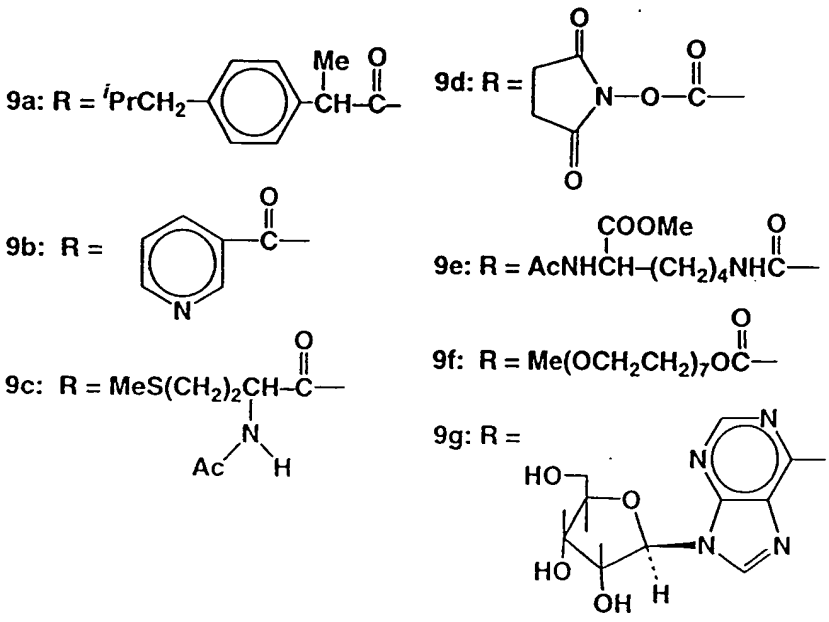
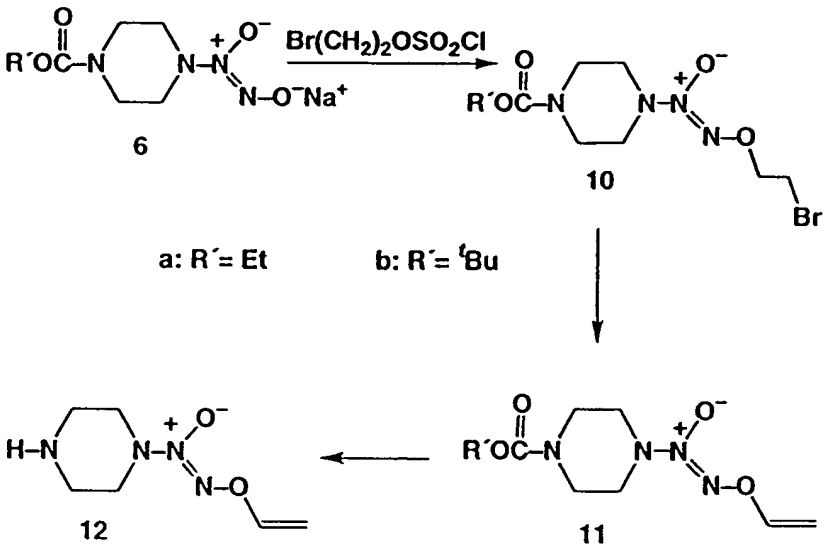


Scheme 1. Synthesis of Anionic Piperazine Diazeniumdiolates

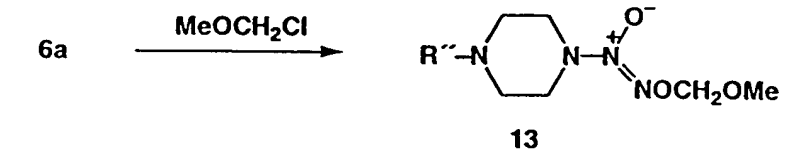




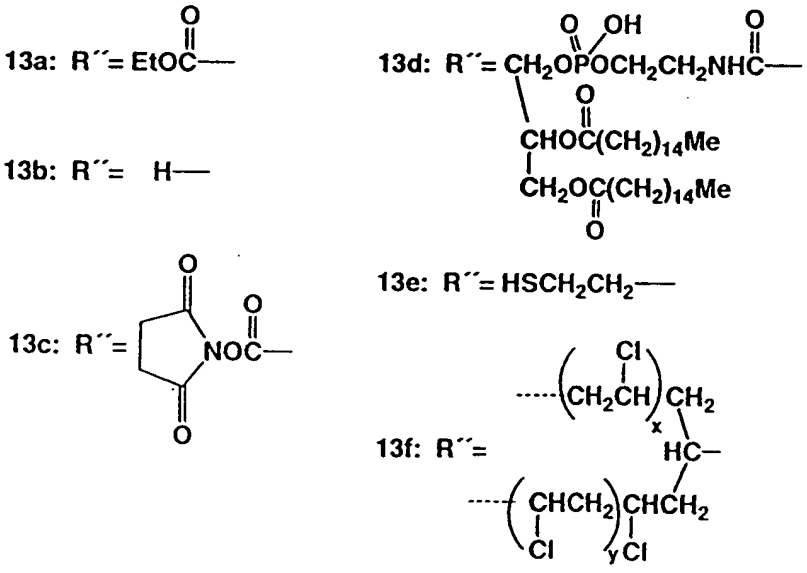
Scheme 2. Synthesis of O^2–Methylated Piperazine Diazeniumdiolates



Scheme 3. Synthesis of O^2–Vinylated Piperazine Diazeniumdiolates



13



Scheme 4. Synthesis of O^2–Methoxymethylated Piperazine Diazeniumdiolates

SUPPORTING INFORMATION

for

Piperazine as a Linker for Incorporating the Nitric Oxide-releasing Diazeniumdiolate Group into Other Biomedically Relevant Functional Molecules

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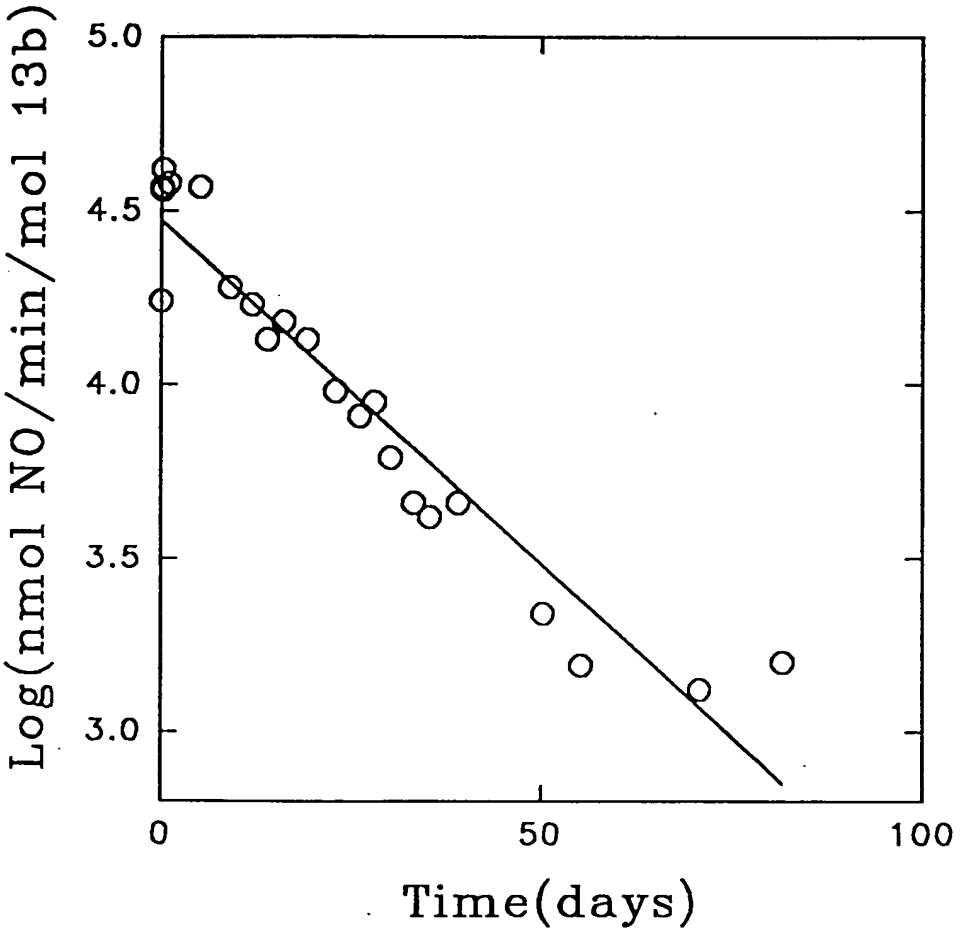
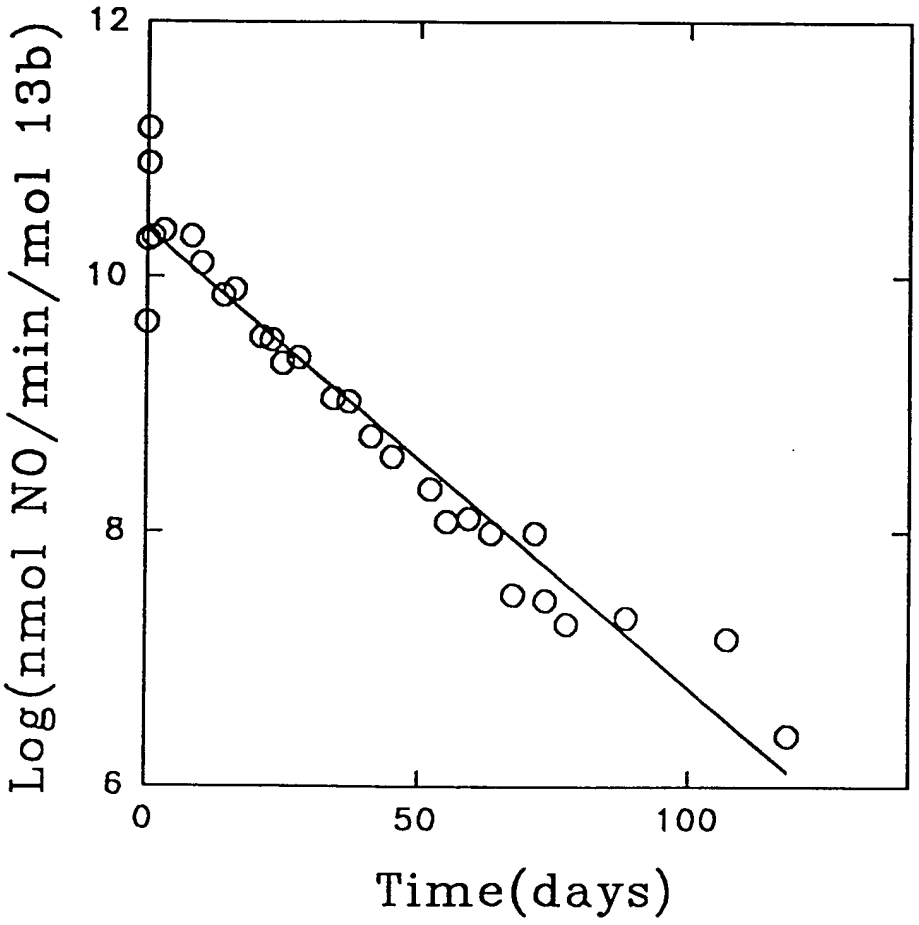
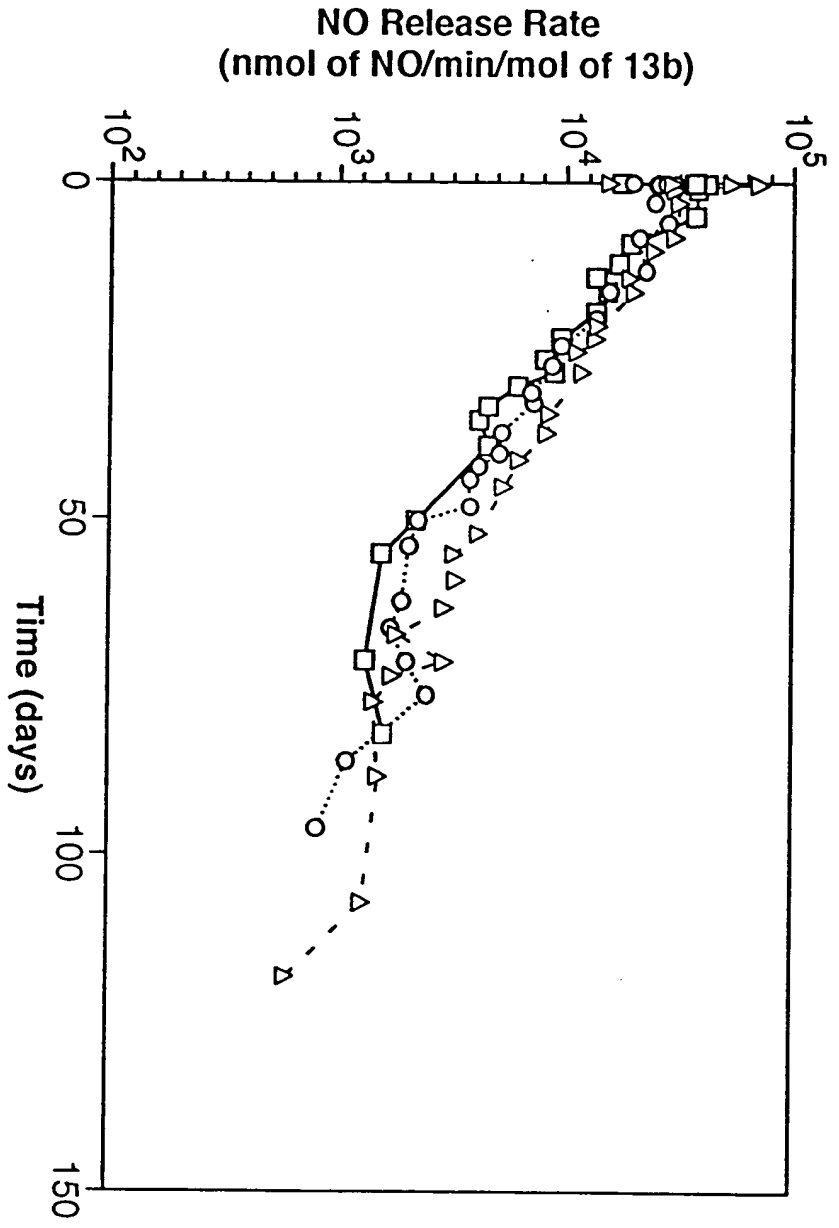
Contents


Log(nmol NO/min/mol 13b)
5.0
4.5
4.0
3.5
3.0
0
50
100
Time(days)


Log(nmol NO/min/mol 13b)
12
10
8
6
0
50
100
Time(days)

(1)


NO Release Rate
(nmol of NO/min/mol of 13b)
10^2
10^3
10^4
10^5
0
50
100
150
Time (days)